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Original article

Application of *Beauveria bassiana* using different baits for the control of grasshopper pest *Dichroplus maculipennis* under field cage conditions

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ABSTRACT

Dichroplus maculipennis is a widely distributed species, occurring in several countries of southern South America. Poisoned baits are effective for the control of insect pests. Adding attractants and phagostimulants could result in improved bait formulations, making bait treatment even more efficacious, for the control of grasshopper pests. The goal of the study was to determine, under laboratory bioassay and field cage conditions, the most effective treatment option using different baits with the entomopathogenic fungi *Beauveria bassiana*, for the control of the grasshopper pest *D. maculipennis*. In laboratory bioassays we observed significant differences (df = 11; f = 2.23; $p < 0.01$) in percentages of mortality against third-instar nymphs of *D. maculipennis* caused by different strains of *B. bassiana*, as well as between the different treatments studied. Under field cage conditions, the highest mortality was 100% in treatment 3 (canola oil, wheat bran, and conidia) and the lowest was 73.3 in treatment 1 (conidia only). The lowest value of median survival time (MST) was obtained with the combination LPSc 1227 conidia plus wheat bran and canola oil (6.43 d). In the laboratory bioassays and under field cage conditions the combination of conidia of *B. bassiana* with wheat bran and canola oil improved the performance of conidia, enhancing mortality of *D. maculipennis*.

1. Introduction

The univoltine and polyphagous grasshopper *Dichroplus maculipennis* (Blanchard) (Orthoptera: Acrididae) is a widely distributed species, occurring in Brazil, Argentina, Chile and Uruguay (Carbonell et al., 2017). It is also one of the most damaging grasshoppers in Argentina, particularly in large areas of the Pampas and Patagonia regions (Mariottini et al., 2013; Cigliano et al., 2014). From late 2008 through early 2011 a major outbreak of *D. maculipennis* occurred in the southern Pampas. Densities reached up to 75 individuals per m^2, swarm-like aggregative dispersal flights were observed, and crops (corn, soybean, sorghum, wheat, barley, alfalfa, wheat-grass, ray-grass, clover) and natural grasslands in an area of approximately 2.5 million ha were affected (Mariottini et al., 2012). Currently, chemical insecticides are the only option for the control of *D. maculipennis* but the use of these chemicals pollutes the environment and brings great problems in the health of farmers (Álvarez et al., 2013). The requirement for nonchemical alternatives has become increasingly important in recent years (Foster et al., 2010). *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin s.l. is commonly used for the biological control of insect pests of agricultural crops (Jaronski, 2010; Vega et al., 2012). Insecticidal baits have been used for the control of acridid pests worldwide for more than a century. Baits are used on a regular basis in North America to control rangeland grasshoppers and Mormon crickets (*Anabrus simplex* Haldeman, Orthoptera: Tettigoniidae) (Latchininsky et al., 2007). A typical bait of these formulations consists of wheat bran or other solid carrier impregnated with a chemical or biological insecticide. An advantage of using baits might be protection of the fungal pathogen from degradation due to UV light and/or other environmental factors. Adding

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attractants and phagostimulants could result in improved bait formulations, making bait treatment even more efficacious (Latchininsky and VanDyke, 2006). Vegetable oils have kairomonal attractant properties for grasshoppers primarily due to the presence of linoleic and linolenic fatty acids (Bomar and Lockwood, 1994a). These fatty acids are dietary essentials for grasshoppers and, once volatilized, can be detected by the insects' olfactory chemical-receptors (Bomar and Lockwood, 1994b). Due to the presence of fatty acids, certain vegetable oils used as insecticide carriers, can function as liquid baits, and markedly enhance the efficacy of grasshopper control programs (Lockwood et al., 2001; Latchininsky et al., 2007). The aim of this study was to determine, under laboratory bioassay and field cage conditions, the most effective treatment option (spray or bait) with the entomopathogenic fungus *B. bassiana*, for the control of the grasshopper pest *D. maculipennis*. The objectives were: 1) evaluate three *B. bassiana* strains for potential use against *D. maculipennis* nymphs; 2) compare spray and bait formulations of each *B. bassiana* strains against *D. maculipennis* nymphs in laboratory bioassays; and 3) evaluate spray and bait formulations of the *B. bassiana* strain that performed best in laboratory bioassays under field cage conditions against *D. maculipennis* nymphs.

2. Materials and methods

2.1. Insect

Nymphs of *D. maculipennis* used in this study belonged to the first laboratory generation [F1] of specimens originally collected in the southern Pampas region, Argentina. The nymphs were maintained kept at (30 °C, photoperiod 14–10 h L-D, 40% RH) according to Mariottini et al. (2011).

2.2. Fungal isolate

The three fungal strains of *B. bassiana* used in this study were isolated of the locust *Schistocerca cancellata* (Serville). Cadavers of *S. cancellata* were collected near La Banda (27° 44′ 07″ S; 64° 14′ 36″ W), Santiago del Estero province, Argentina. These strains were deposited at the Spegazzini Institute culture-collection with the following access numbers: LPSc 1225; LPSc 1226; LPSc 1227 (accession Gen Bank numbers MG012790, MG012791, and MG012792).

2.3. Spray and bait formulation

Conidia from *B. bassiana* strains were obtained according to Pelizza et al. (2017). Conidia were harvested and placed in test tubes containing 0.01% (v/v) polyoxyethylene sorbitan monolaurate (Tween 80™; Merck) and Extreme Rizospray (Rizobacter®) concentrations according to suggestions by the manufacturer, at a rate of 500 μl per liter of product as adjuvant (silicon organ plus refined and modified vegetable oil) in all treatments. Suspensions were adjusted to 1×10^8 conidia mL^{-1} using a Neubauer haemocytometer. Viability of conidia used in the tests was determined after 24 h as described by Goettel and Inglis (1997).

2.4. Laboratory bioassays

Five treatments were performed (Table 1). In all cases, three replicates (on different dates) of 10 third-instar *D. maculipennis* nymphs were used and the grasshoppers were kept in wire-screened, aluminium cages (30 × 20 × 20 cm). Both control and treated grasshoppers were placed under controlled conditions of temperature, relative humidity and photoperiod (26 °C, 60%, and 14:10-h L:D). The mortality of the nymphs of *D. maculipennis* was recorded systematically each 24 h for ten days. Mycosis was confirmed by microscopic optical examination of dead grasshoppers.

2.5. Trial under field cage conditions

The field trial was carried out with the strain of *B. bassiana* that showed higher mortality in each of the different treatments under laboratory conditions. It was conducted during December 2016 at a field near Tandil, located in the southeast of the Buenos Aires province (37°19′00″ S; 59°08′60″ W). The pastures that were mainly in the field were *Bromus* and *Lolium*. The same five treatments were carried out as in the laboratory tests (Table 2), but in this field trial treated and control nymphs were placed in wire-screened, floorless, aluminium cages (70 × 50 × 50 cm), so insects were allowed to feed on natural grasses under natural conditions. Twenty third-instar *D. maculipennis* nymphs were placed inside each cage. A manual 2-liter sprinkler was used to perform the conidial spraying of *B. bassiana* combined with the adjuvant (Extreme Rizospray) or canola oil. In all cases, three replicates (on different dates) of 20 third-instar *D. maculipennis* nymphs were used. The cumulative mortality was recorded daily for 15 d. Dead grasshoppers with no external mycelium were surface-sterilized by dipping them successively in 70% ethanol (10–15 s), 0.5% sodium hypochlorite solution (1 min), and sterile distilled water (1 min, two consecutive baths) according to Lacey and Brooks (1997). Next, they were placed in a sterile culture chamber consisting of a Petri dish (60 mm diameter) with a filter-paper disk that was periodically moistened with sterile distilled water and incubated at 25 °C in the absence of light. Mycosis was confirmed by microscopic examination of dead grasshoppers. Maximum and minimum temperatures and relative humidity was recorded daily in the site where the test was performed. The average temperature registered during the field trials fluctuated between a maximum of 33 °C and a minimum of 17.4 °C, and the relative humidity averaged was 47%.

Table 1
Treatments carried out in laboratory bioassays.

Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Third-instar *D. maculipennis* nymphs were sprayed with 3 ml of each strain suspension containing 1×10^8 conidia/ml with a glass atomizer.	Two hundred grams of wheat bran were sprinkled with a suspension of 1×10^8 conidia/ml of *B. bassiana*. This bait was introduced into wire-screened aluminium cages housing 10 third-instar *D. maculipennis* nymphs. This procedure was carried out for each of the strains studied.	Third-instar *D. maculipennis* nymphs were sprayed with 3 ml of each strain suspension containing 1×10^8 conidia/ml plus canola oil with a glass atomizer.	Two hundred grams of wheat bran were sprinkled with a suspension of 1×10^8 conidia/ml of *B. bassiana* plus canola oil. This bait was mixed and then was introduced into wire-screened aluminium cages housing 10 third-instar *D. maculipennis* nymphs. This procedure was carried out for each of the strains of *B. bassiana* studied.	Third-instar *D. maculipennis* nymphs were sprayed with 1 ml of adjuvant (Extreme rizospray) only. In addition, the wheat bran mixed with the canola oil but without the addition of the fungal inoculum was offered to the grasshoppers for consumption.

Table 2
Treatments carried out under field cage conditions.

Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Third-instar *D. maculipennis* nymphs were sprayed with suspension containing 1×10^8 conidia/ml through the use of a manual 2-l sprinkler.	Two hundred grams of wheat bran sprayed with a suspension of 1×10^8 conidia/ml of *B. bassiana* were dispersed, on the soil of each of the cages.	Nymphs were sprayed with a suspension of 1×10^8 conidia/ml that had the adjuvant (Extreme Rizospray) at a rate of 0.5 ml *per* liter of formulation, but unlike treatment 1, canola oil was added to the suspension.	Two hundred grams of wheat bran inoculated with a suspension of 1×10^8 conidia/ml of *B. bassiana* were dispersed on the soil of each cage as treatment 2, but here the wheat bran was sprinkled with canola oil.	Third-instar *D. maculipennis* nymphs, used as positive controls, were sprayed in the same fashion but with 1 ml of adjuvant (Extreme Rizospray) only. In addition, the wheat bran mixed with the canola oil but without the addition of the fungal inoculum was placed on the floor of each cage so that the grasshoppers consume it.

2.6. Data analysis

When mortality in treated grasshoppers was 50% or higher the median survival time (MST) was calculated based on the Kaplan-Meier Survival distribution function (Xlstat life software, 2013). Pairwise comparisons between survival curves were made by log-rank test. Significant differences between percent mortalities after a 10-day exposure to the different treatments were analysed using one-way analysis of variance (ANOVA). For later comparisons the Tukey test ($p = 0.05$) was used. Analyzes of variance were performed with the InfoStat 2007 software (InfoStat, 2001).

3. Results

In the laboratory bioassays was observed significant differences (df = 11; f = 2.23; $p < 0.01$) in the percentages of mortality against third-instar nymphs of *D. maculipennis* caused by the strains of *B. bassiana*, as well as between the treatments assessed. Was observed that isolate LPSc 1227 caused the highest mortality at each treatments tested, ranging from 93.3% in the treatment 1 (conidia only) to 100% in the treatments 2 (Wheat bran with conidia) and 3 (Canola oil, wheat bran, and conidia) (Fig. 1). On the other hand, the strain LPSc 1226 caused lower mortality, at each treatment tested, ranging from 73.3% in treatment 3 (Canola oil with conidia) to 100% in treatment 4 (Canola oil, wheat bran, and conidia) (Fig. 1). In the laboratory bioassays the MST all three strains in the treatment 1 (conidia only), showed that LPSc 1227 was the most virulent with values of 6 d while the least virulent isolate was LPSc 1226 with 8 d (Table 1). Moreover, the lower value of MST was obtained with the combination LPSc 1227 conidia-wheat bran plus canola oil (3.2 d) and the highest MST was observed in the combination LPSc 1226 conidia plus wheat bran

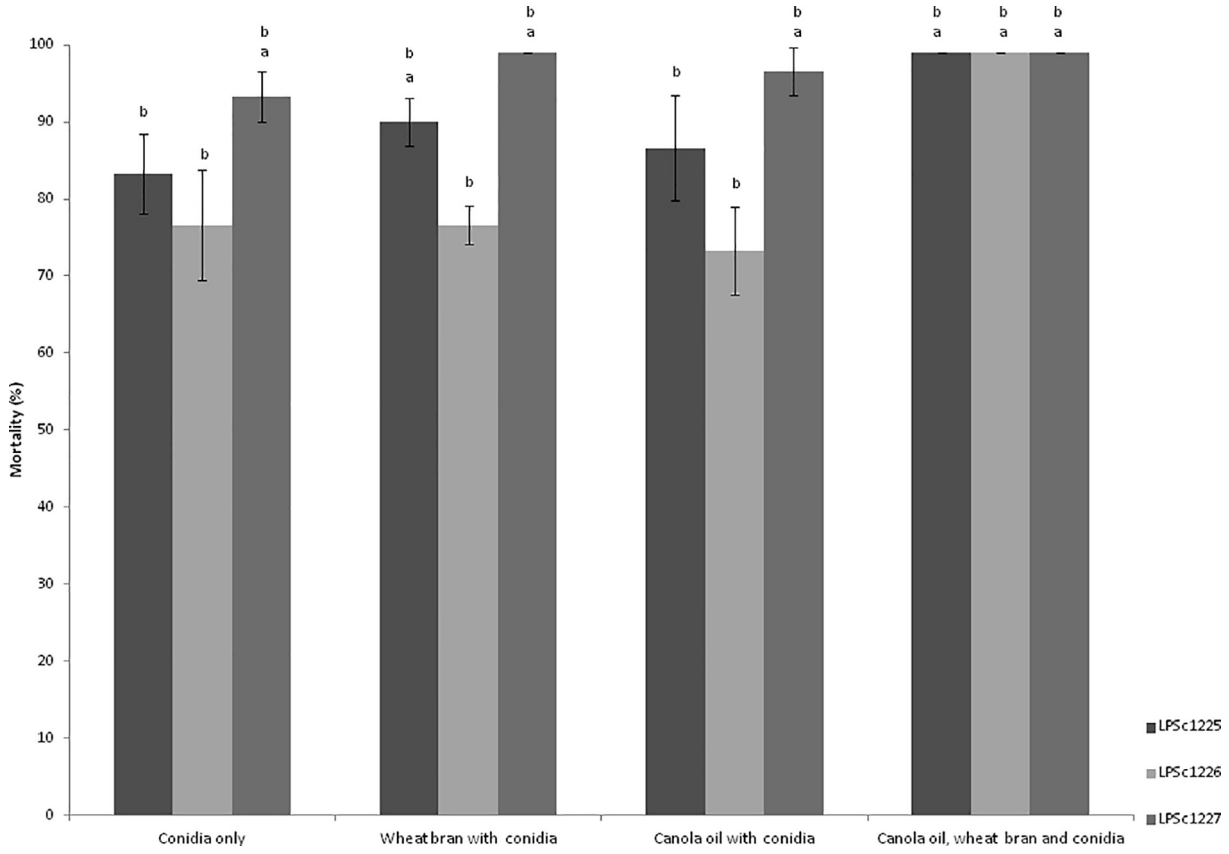

Fig. 1. Cumulative mortality (%) ± SE of third-instar *Dichroplus maculipennis* nymphs within 10 days after infection with 1×10^8 conidia/ml of *Beauveria bassiana* strains (LPSc 1225; LPSc 1226 and LPSc 1227) under laboratory conditions. Different letters denote significant differences between *Beauveria bassiana* strains in the same treatment, according to the Tukey test ($p < 0.05$).

Table 3
Median Survival Time in days (±SE) of third-instar *D. maculipennis* nymphs, when they were sprayed or fed with baits poisoned with the different strains of *B. bassiana* LPSc 1225; LPSc 1226 and LPSc 1227 alone and in combination with canola oil, wheat bran and canola oil plus wheat bran. Different letters denote significant differences between treatments in the same column according to the Log-rank test ($p < 0.05$).

Strains (LPSc)	Median Survival Time			
	Conidia only	Conidia with canola oil	Wheat bran with conidia	Canola oil and wheat bran with conidia
1225	6.5 ± 0.56 b	6.5 ± 0.53 b	7.5 ± 0.45 a	4.2 ± 0.39 b
1226	8 ± 0.35 a	7.4 ± 0.44 a b	7.9 ± 0.36 a	6 ± 0.41 a
1227	6 ± 0.46 b	4.8 ± 0.49 c	5.5 ± 0.53 b	3.2 ± 0.29 b

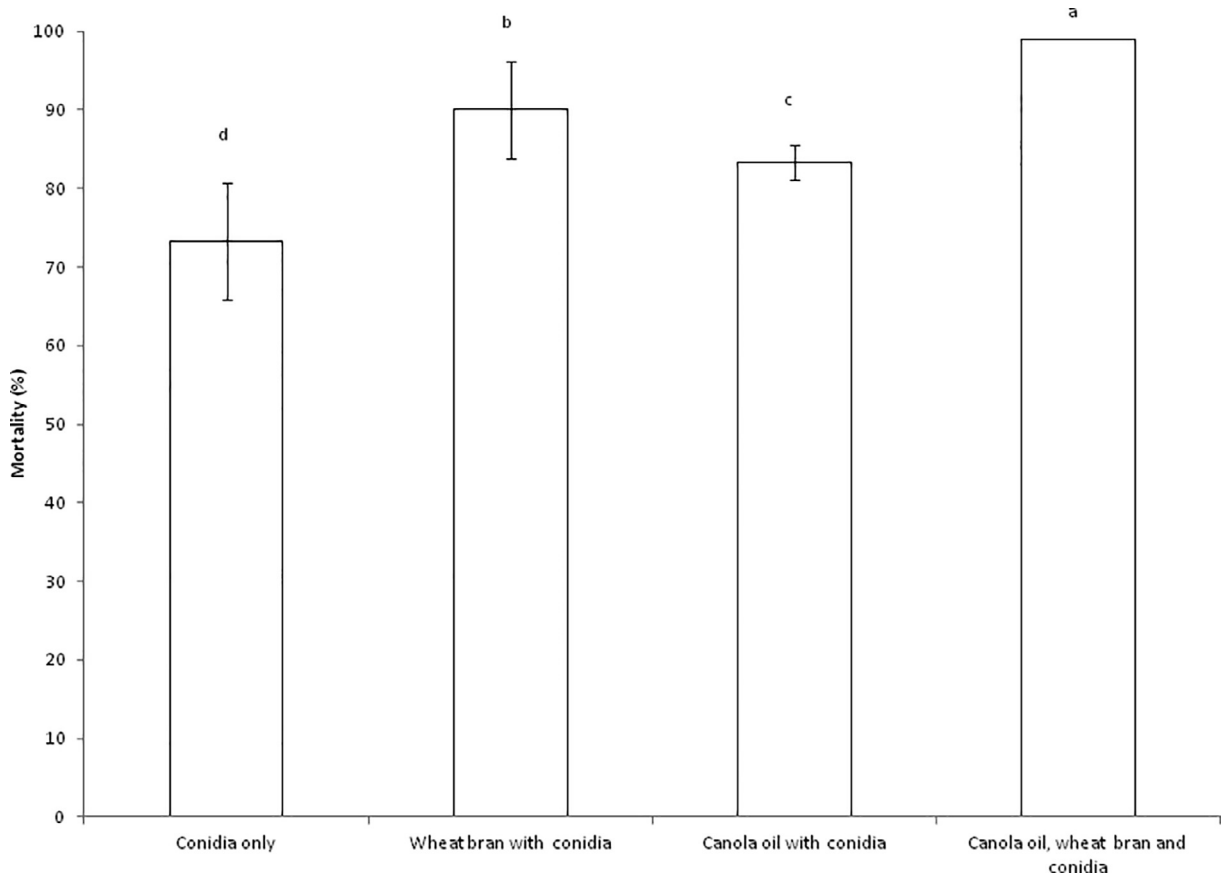

Fig. 2. Cumulative mortality (%) ± SE of third-instar *Dichroplus maculipennis* nymphs within 15 days after infection with 1×10^8 conidia/ml of LPSc 1227 *Beauveria bassiana* strain, under cage field conditions. Different letters denote significant differences between treatments, according to the Tukey test ($p < 0.05$).

(7.9 d) (Table 3). Under field cage conditions, significant differences were observed (df = 3; f = 328.67; $p < 0.0001$) in percentages of mortality against third-instar nymphs of *D. maculipennis* caused by LPSc 1227 strain of *B. bassiana* between the different treatments. The highest mortality was 100% in treatment 3 (canola oil, wheat bran, and conidia) and the lowest was 73.3 ± 2.2% in treatment 1 (conidia only) (Fig. 2). In the field cage trial, the lowest value of MST was obtained with the combination LPSc 1227conidia plus wheat bran and canola oil (6.4 d), and the highest MST was in treatment 1 (conidia only) at 8.3 d. Intermediate values of MST were observed with the combination LPSc 1227-wheat bran at 7.4 d and with LPSc 1227-canola oil at 7 d. No mortality was observed in the control (treatments 5), either in laboratory bioassays as in the field cage trial.

4. Discussion

In this work, we found that in the laboratory bioassays and under field cage conditions the combination of conidia of all three *B. bassiana* strains with wheat bran and canola oil improved the performance of conidia, enhancing mortality of *D. maculipennis* nymphs. In addition, we observed a reduction in MST when conidia of the different *B. bassiana* strains were combined with wheat bran and canola oil. Both canola oil and wheat bran likely provided protection to conidia against UV rays from solar radiation, allowing propagules to remain viable longer and thus causing higher percentage of infection on *D. maculipennis* nymphs. Studies carried out by Latchininsky et al. (2007) reported that there are certain vegetable oils such as olive, canola, corn, and flax that have phagostimulant properties on nymphs and adults of the North American grasshopper pest *Melanoplus sanguinipes* (Fabricius). That *D. maculipennis* nymphs were attracted to wheat bran baits impregnated with canola oil in our tests both in the laboratory and under field cage conditions would agree with Latchininky's reports for *M. sanguinipes* in light of the relatively close phylogenetic relationship between melanoplines of genera *Melanoplus* and *Dichroplus* (Song et al., 2018). The use of poisoned baits with different chemical insecticides for the control of species of grasshopper pests is not a novelty (Onsager et al., 1980; Erickson and Onsager, 1981; Cowan, 1990). In the USA they have

been used extensively and frequently, including as the main control mechanism of the once most noxious and now possibly extinct grasshopper *Melanoplus spretus*. To control this grasshopper pest and others, the bait called "Criddle mixture" was widely used (3000 t over 500.000 ha in Kansas alone in 1917; Lockwood, 2004), which was manufactured with dry horse dung and then sprayed with chemical insecticides. Baits are already being used successfully since 1980 successfully for standard application of spores of the long-term biocontrol agent of grasshoppers *Paranosema locustae* (Microsporidia) (Solter et al., 2012; Bjornson and Oi, 2014; Lange and Sokolova, 2017) and have been also employed for small experimental applications of *Malameba locustae* (Rhizopoda) (Lange and Lord, 2012). On the contrary, a work of Foster et al. (2010) showed that the use of baits with conidia of *B. bassiana* and *Metarhizium brunneum* Petch used for the control of the Mormon cricket *Anabrus simplex* (Haldeman), did not yield satisfactory results. One possible explanation for this result could have been that these baits were sprayed with paraffin oil instead of a vegetable oil such as olive or canola, rendering them less palatable. In several studies carried out under field conditions, in which entomopathogenic fungi were used for the control of acridid species, the mortality percentages obtained ranged between 70 and 90% after 9–20 d (Lomer et al., 2001; Magalhães et al., 2000; Peng et al., 2008; Keyser et al., 2017). In this work, we observed the highest mortality and the lowest MST values, both in the laboratory tests and those performed under field cage conditions.

5. Conclusions

In this work it was demonstrated that the combination of canola oil with wheat bran makes the bait poisoned with conidia of *B. bassiana* more palatable for nymphs of *D. maculipennis*. This was reflected in the higher mortality and lower MST values for this species of grasshoppers pest, both in laboratory bioassays and those carried out under field cage conditions. Furthermore, the use of this kind of bait has an important advantage respecting to the conventional application (spray), as biological insecticides are commonly applied under field conditions. This advantage is due to wheat bran protects the conidia of the entomopathogenic fungus from the direct action of radiation UV, and also, the canola oil, allows the conidia to stay hydrated for a more time, allowing it to maintaining its pathogenicity for more time. Although it would be necessary to make a thorough evaluation of the economic costs involved in the production of this type of bait for the control of *D. maculipennis*, we think that it could be a viable alternative that may contribute to minimize as much as possible the current use of chemical insecticides.

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